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Dating the impossible: the origin and divergence of the largest infection on Earth

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Wolbachia are common intracellular bacteria of many arthropod and nematode species; they are responsible for likely the most widespread and evolutionary significant infection on Earth. *Wolbachia* are generally acquired by maternal transmission, but they often move across species boundaries, impairing the use of host divergences as independent calibration to infer their evolutionary history. Here we present a first systematic effort to estimate the origin and the divergence of the *Wolbachia* infection by coupling the molecular clock analysis of a genomic and an MLST data sets with three types of disputed calibration priors: a root prior for splits within Proteobacteria, the recently proposed *Nomada* host-symbiont co-divergence, and an adjusted mutation rate gathered from *Drosophila melanogaster* and *Nasonia* species. We first perform a hierarchical based model comparison on the MLST data set to select the best clock, replacement, and tree priors; we then used the best prior combination to calibrate both the genomic and the MLST data sets using different combinations of calibrations. Although divergence estimates are characterized by a high degree of uncertainty and strongly depend on the use of certain calibrations, most results point towards a pre-Cambrian origin of *Wolbachia* (its split from *Ehrlichia*). Diversification of *Wolbachia* supergroups is more recent, from the late Paleozoic to the Mesozoic: this is compatible with a long-fuse model of evolution, and suggests a co-radiation with holometabolan insects. Our results provide a comprehensive first effort to date *Wolbachia* evolution: results are model, prior, and data set dependent and reveal the difficulties embedded in the molecular dating of such elusive bacteria characterized by the absence of reliable calibration priors and a clear evolutionary history.